

U.S. Patent Application No. 10/522,000  
Amendment dated May 22, 2008  
Reply to Final Office Action of November 26, 2007

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**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (Currently amended) A labeled single chain antibody having a structure in which a heavy chain and a light chain of an antibody are directly crosslinked through a linker, wherein ~~the~~ the linker is bound to a labeling substance, wherein the linker comprises an amino acid sequence that is recognizable by a biotin ligase, and wherein the biotin ligase binds the labeling substance to the linker.
2. (Currently amended) The labeled single chain antibody of claim 1, ~~carrying a labeling substance in a linker part of a single chain antibody,~~ wherein a the heavy chain and a the light chain of the antibody are variable regions.
3. (Canceled).
4. (Canceled).
5. (Currently amended) ~~The A~~ A labeled single chain antibody ~~of claim 1,~~ having a structure in which a heavy chain and a light chain of an antibody are crosslinked through a linker, ~~and carrying a labeling substance in the linker part,~~ wherein the linker comprises a labeling substance, and wherein the labeling substance is incorporated as one part of the linker part of the antibody.
6. (Currently amended) The labeled single chain antibody of claim ~~1~~ 5, having a structure in which a the heavy chain and a the light chain that are variable regions of the antibody are crosslinked through a the linker, ~~and carrying a labeling substance in the linker part,~~ ~~wherein the labeling substance is incorporated as one part of the linker part of the antibody.~~

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7. (Currently amended) The labeled single chain antibody of claim 1, having a structure in which a the heavy chain and a the light chain of the antibody are crosslinked through a the linker, ~~and carrying in the linker part a labeling substance that is capable of binding to a polypeptide of the linker part of the antibody in the presence of a specific enzyme~~, wherein the labeling substance is biotin ~~and the enzyme is a biotin ligase~~.

8. (Canceled)

9. (Currently amended) The labeled single chain antibody according to claim 1, which has a Kd value that is equivalent to a Kd value of a ~~naturally occurring~~ parental antibody and which is produced by a cell-free protein translation system using wheat embryo.

10-19. (Canceled)

20. (Currently amended) A labeled single chain antibody which has a Kd value that is equivalent to a Kd value of a parental antibody and is produced by a method for producing a labeled single chain antibody wherein DNA is subjected to transcription and translation, utilizing a wheat embryo-derived cell-free protein translation system in the presence of a labeling substance and in the presence of an enzyme that catalyzes a disulfide bond exchange reaction, and wherein the DNA comprises DNAs encoding a heavy chain and a light chain of an antibody having binding ability against a specific antigen, wherein the heavy chain and the light chain that are linked through a DNA encoding a linker, wherein the DNA encoding a linker comprises a nucleotide sequence that is capable of binding with a labeling substance in the presence of a specific enzyme after translation wherein the linker comprises an amino acid sequence that is recognizable by a biotin ligase, and wherein the biotin ligase binds the labeling substance to the linker.

21. (Currently amended) A method for producing an immobilized single chain antibody,

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wherein any one of the antibodies described hereunder is brought into contact with a reaction plate compartmentalized into a plurality of regions having on the surface thereof a substance that binds specifically with a labeling substance of the antibody:

~~1) — a labeled single chain antibody of claim 1, wherein the antibody has a structure in which a heavy chain and a light chain of the antibody are crosslinked through a linker and the linker is bound to a labeling substance in the linker part;~~

~~2) — a labeled single chain antibody having a structure in which a heavy chain and a light chain of the antibody are crosslinked through a linker, and the linker is bound to a labeling substance, wherein the heavy chain and the light chain of the antibody are variable regions;~~

~~3) a labeled single chain antibody having a structure in which a heavy chain and a light chain of the antibody are crosslinked through a linker, and the linker is bound to a labeling substance, wherein the labeling substance is a substance that is capable of binding to a polypeptide of the linker part of the antibody in the presence of a specific enzyme, wherein the linker comprises an amino acid sequence that is recognizable by a biotin ligase, and wherein the biotin ligase binds the labeling substance to the linker;~~

~~[[4]]2) a labeled single chain antibody having a structure in which a heavy chain and a light chain that are variable regions of the antibody are crosslinked through a linker, and the linker is bound to a labeling substance, wherein the labeling substance is a substance that is capable of binding to a polypeptide of the linker part of the antibody in the presence of a specific enzyme, wherein the linker comprises an amino acid sequence that is recognizable by a biotin ligase, and wherein the biotin ligase binds the labeling substance to the linker;~~

~~5) — a labeled single chain antibody having a structure in which a heavy chain and a light chain of the antibody are crosslinked through a linker, and the linker is bound to a labeling~~

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~~substance, wherein the labeling substance is incorporated as one part of the linker part of the antibody;~~

~~6) a labeled single chain antibody having a structure in which a heavy chain and a light chain that are variable regions of the antibody are crosslinked through a linker, and the linker is bound to a labeling substance, wherein the labeling substance is incorporated as one part of the linker part of the antibody;~~

73) a labeled single chain antibody having a structure in which a heavy chain and a light chain of the antibody are crosslinked through a linker, and the linker is bound to a labeling substance that is capable of binding to a polypeptide of the linker part of the antibody in the presence of a specific enzyme wherein the linker comprises an amino acid sequence that is recognizable by a biotin ligase, wherein the biotin ligase binds the labeling substance to the linker and, wherein the labeling substance is biotin ~~and the enzyme is a biotin ligase;~~

84) a labeled single chain antibody having a structure in which a heavy chain and a light chain that are variable regions of the antibody are crosslinked through a linker, and the linker is bound to a labeling substance ~~that is capable of binding to a polypeptide of the linker part of the antibody in the presence of a specific enzyme~~ wherein the linker comprises an amino acid sequence that is recognizable by a biotin ligase, wherein the biotin ligase binds the labeling substance to the linker and, wherein the labeling substance is biotin ~~and the enzyme is a biotin ligase.~~

22. (Original) The method for producing an immobilized single chain antibody of claim 21, wherein two or more kinds of different immobilized single chain antibodies are immobilized on a reaction plate compartmentalized into a plurality of regions.

23. (Previously presented) The production method according to claim 21, wherein a labeling

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substance is biotin and a substance that binds specifically with the labeling substance is streptavidin.

24. (Previously presented) An immobilized single chain antibody prepared by the production method according to claim 21.

25. (Withdrawn) A method for analyzing an antigen-antibody reaction, wherein a test substance is brought into contact with the immobilized single chain antibody of claim 24, and binding ability of the test substance against the immobilized single chain antibody is analyzed.

26. (Withdrawn) A method for analyzing an antigen-antibody reaction, comprising the steps of:

(1) preparing a labeled single chain antibody under conditions in which a disulfide bond of a single chain antibody is retained, comprising the step of the following (i) or (ii):

(i) producing a labeled single chain antibody by subjecting a DNA, in which DNAs encoding a heavy chain and a light chain of an antibody having binding ability with a specific antigen are linked through a DNA encoding a linker comprising a nucleotide sequence that is capable of binding with a labeling substance in the presence of a specific enzyme after translation, to transcription and translation utilizing a wheat cell-free protein synthesis system in the presence of a specific enzyme; or

(ii) producing a labeled single chain antibody by subjecting a DNA, in which DNAs encoding a heavy chain and a light chain that are variable regions of an antibody having binding ability with a specific antigen are linked through a DNA encoding a linker comprising a nucleotide sequence that is capable of binding with a labeling substance in the presence of a specific enzyme after translation, to transcription and translation utilizing a wheat cell-free protein synthesis system in the presence of a specific

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enzyme;

(2) preparing a substance (adapter substance) that binds specifically with a labeling substance of a labeled single chain antibody in a case where the labeling substance of the labeled single chain antibody is an immobilizing substance, comprising the steps of:

(i) immobilizing a substance (adapter substance) that binds specifically with a labeling substance of a labeled single chain antibody to a reaction plate compartmentalized into a plurality of regions;

(ii) removing a substance (adapter substance) that binds specifically with a labeling substance of a labeled single chain antibody that was not immobilized to the reaction plate in the preceding (i); and

(iii) before and after the step of the preceding (i) or (ii), removing nonspecific adsorption from the reaction plate as appropriate;

(3) preparing an immobilized labeled single chain antibody in a case where a labeling substance of the labeled single chain antibody is an immobilizing substance, comprising the steps of:

(i) adding a required amount of the labeling substance of the labeled single chain antibody prepared in (i) or (ii) of the above (1) onto a reaction plate compartmentalized into a plurality of regions having a substance (adapter substance) of (2) that binds specifically with the labeling substance of the labeled single chain antibody on the surface thereof, whereby to contact;

(ii) removing a labeled single chain antibody that was not immobilized to the substance (adapter substance) that binds specifically to the labeled single chain antibody on the reaction plate in the preceding (i); and

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- (iii) following the preceding step (ii), removing nonspecific adsorption from the reaction plate as appropriate;
  - (4) preparing a labeled single chain antibody in a case where a labeling substance is a signal substance, comprising the steps of:
    - (i) removing nonspecific adsorption from a reaction plate compartmentalized into a plurality of regions as appropriate; and
    - (ii) adding a required amount of the labeling substance of the labeled single chain antibody prepared in (i) or (ii) of the above (1) onto the reaction plate;
  - (5) adding a required amount of a test substance onto each reaction plate according to the above (3) or (4), and analyzing the binding ability of a labeled single chain antibody with the test substance; and
  - (6) based on the binding ability result obtained in the above (5), qualitatively or quantitatively determining the interaction between the labeled single chain antibody and the test substance.
27. (Withdrawn) A reagent kit for measuring an antigen-antibody reaction, comprising a reagent to be used in the analysis method according to claim 25.
28. (Previously presented) An immobilized single chain antibody that has a  $K_d$  value that is equivalent to a  $K_d$  value of a parental antibody and that is produced by the method for producing an immobilized single chain antibody according to claim 21 utilizing a wheat embryo-derived cell-free protein translation system.
29. (New) A labeled single chain antibody which has a  $K_d$  value that is equivalent to a  $K_d$  value of a parental antibody and is produced by a method for producing a labeled single chain antibody wherein DNA is subjected to transcription and translation, utilizing a wheat embryo-

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derived cell-free protein translation system in the presence of a labeling substance and in the presence of an enzyme that catalyzes a disulfide bond exchange reaction, and wherein the DNA comprises DNAs encoding a heavy chain and a light chain of an antibody having binding ability against a specific antigen, wherein the heavy chain and the light chain are linked through a DNA encoding a linker, wherein the linker comprises a labeling substance, and wherein the labeling substance is incorporated as one part of the linker part of the antibody.